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Behavioral and Hormonal Changes in Female Naked Mole-Rats (*Heterocephalus glaber*) Following Removal of the Breeding Female from a Colony

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Colonies of naked mole-rats (*Heterocephalus glaber*) contain a single dominant, breeding female, or "queen," which suppresses reproduction in subordinate females. In this study, the queen and breeding male were removed from a colony at Brookfield Zoo. We examined behavioral and endocrine changes in the remaining colony females. Behavioral observations were conducted weekly and urine samples were collected beginning 3 months prior to the planned removal of animals. Prior to the removal, only the queen displayed the high frequencies of aggressive shoving typical of breeding females. In the first 2 months following removal of the queen, three non-breeding females markedly increased their frequencies of shoving. Urinary progesterone showed that one of these three females had probably ovulated both before and after queen removal. Histological examination following fatal agonistic interactions confirmed ovulatory function in two of these three females. Similar behavioral and endocrinological results were obtained for three more females, following the combat deaths of the first three females. These findings suggest that ovarian activation facilitates intrasexual aggression in female naked mole-rats and might contribute to attainment of breeding status.

Naked mole-rats (*Heterocephalus glaber;* family Bathyergidae) are subterranean, hystricomorph rodents that inhabit arid regions of East Africa. The species is unique among mammals in that colonies, which may contain up to several hundred animals, have only a single breeding female and one to three breeding males. All other animals in a naked mole-rat colony exhibit reproductive suppression and a division of labor resembling that found in the eusocial insects (Jarvis, 1981; Lacey and Sherman, 1991; Faulkes, Abbott, Liddell, George, and Jarvis, 1991). No other mammal is known to exhibit such an extreme form of sociality and reproductive suppression.

Social suppression of reproduction occurs in a wide variety of organisms

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and may be mediated by behavior (French, Abbott, and Snowdon, 1984; Abbott, 1987; Rhine, Wasser, and Norton, 1988; Abbott, Barrett, Faulkes, and George, 1989; Dunbar, 1989; Creel, Creel, Wildt, and Monfort, 1992). In some species, including the naked mole-rat, specific behaviors performed by one animal (typically the dominant or breeding female) effectively prevent subordinate females from breeding. This type of suppression may involve interference with reproductive behaviors such as mating, thereby preventing subordinate animals from conceiving, or it may disrupt reproductive physiology of subordinate females. Previous studies have shown that subordinate female naked mole-rats fail to ovulate and that the ovulatory block is due to insufficient circulating levels of plasma luteinizing hormone (LH), which, in turn, is probably due to reduced or altered secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus (Faulkes, Abbott, and Jarvis, 1990a; Faulkes, Abbott, Jarvis, and Sherriff, 1990b). Despite this extreme suppression however, non-breeding females are capable of becoming reproductively active. If the breeding female is removed from a colony, or dies, another female usually becomes reproductively active in a short time. Furthermore, when a male and female are removed from a colony and paired, or when a female is removed and housed alone, the female generally becomes reproductively active (Jarvis, 1991a; Faulkes et al., 1990a).

Naked mole-rats are spontaneous ovulators, with an ovarian cycle length of approximately 27–38 days (Faulkes et al., 1991). Behavioral estrus lasts only 2–24 hours, during which time the female's vagina is perforate. At other times, the vagina may or may not be perforate (Jarvis, 1991a). Upon removal from a colony, a non-breeding female may exhibit a perforate vagina in as little as 7 days, and elevated progesterone levels may be detected in as little as 19 days (Faulkes et al., 1991). Shimizu and Margulis (in preparation) observed copulation between a previously non-breeding female and male a day after removal from the parent colony.

Because of the unique reproductive system of the naked mole-rat, and the brief time that it has been studied in captivity, most research thus far has focused on the mechanisms of reproductive suppression. In order to discern these mechanisms, most studies have involved observations of intact colonies with a queen, or of newly paired animals, or of colonies in which the breeder has died unexpectedly. We had the opportunity to collect both behavioral and hormonal data from female naked mole-rats both before and after the planned removal of the breeding animals from a colony. Thus, we could systematically observe the succession of reproductive females in a single colony.

The aim of the present study was to examine the behavioral and hormonal changes that occurred in non-breeding female naked mole-rats during the process of queen succession. Specifically, we addressed three questions:

1. Does the breeding female preferentially direct aggression towards those females most likely to become reproductively active?
2. Do non-breeding females begin to perform those behaviors characteristic of the queen (aggressive shoving and ano-genital nuzzling) before attaining reproductive status?
3. Do any females exhibit ovarian activation prior to the queen's removal?

METHODS

Subjects

The study population was a colony of naked mole-rats maintained at the Brookfield Zoo, Brookfield, Illinois. The colony originally numbered 29 (19 males and 10 females) and was obtained by the zoo in 1990 from a captive colony maintained by Professor Jennifer Jarvis at the University of Cape Town, South Africa. Colony numbers grew quickly; at the beginning of the study in June of 1991, the colony contained 41 animals (20 males and 21 females).

All animals were housed in a public display area in the zoo's Fragile Desert exhibit. The enclosure consisted of a series of 14 plexiglass boxes connected by glass tubes. Boxes measured 25 cm on each side and tube diameter was 5 cm. Total length of glass tube available to the colony was 11.07 m. Two boxes were used as food chambers, and two boxes were used by the animals as toilet chambers to which all animals went to urinate and defecate. The use of toilet chambers has been documented in wild naked mole-rat colonies (Brett, 1991), and in captive colonies, blind-ending chambers (i.e., chambers with a single entrance) are typically selected as toilet chambers (Jarvis, 1991b). One blind-ending chamber was continually used by the animals as a toilet chamber. The location of the second chamber varied and was identified by the presence of feces and soiled bedding. The animals were maintained at approximately 25–30°C and 65–75% humidity. Room lights were kept off except during colony cleaning and maintenance. Low-level illumination (7.5-W white bulbs) of nest boxes was provided to enable zoo visitors to see the animals. The animals were fed once per day, generally between 1100 and 1200. Foods consisted of a variety of root vegetables, whole and chopped; mixed fruit, and cereal. Soiled bedding was removed from toilet chambers and food boxes daily, unless a new litter of offspring was present. To facilitate identification during behavioral observations, each animal was tattooed with a dot code made up of a unique combination of one to six dots. Animals were tattooed on both sides of their bodies.

The ages of most animals were known; however, some animals were known only to be from one of three litters born during a 10-month period in 1990. In order to minimize disruption of the colony, animals were

weighed at intervals of 2–3 months, during routine cleaning of the colony. During these cleanings, all animals were removed from the exhibit temporarily and housed together. Individuals were then removed briefly for weighing and returned to the colony.

Behavioral Observation

Behavioral observations were conducted by a single trained observer and began 78 days prior to the planned removal of the breeding animals. Ten-minute focal observations (Altmann, 1974) were carried out two to three times per week on all females that weighed at least 30 g at the start of this study (N = 12 females, including the queen). Our assumption was that no female weighing less than 30 g was likely to become reproductively active, an assumption supported by previous studies (Jarvis, 1991a). The order of behavioral observation was determined randomly at the start of each observation session. Observations were conducted from 0700 to 1000 and from 1230 to 1630. No data were collected during the period immediately following feeding.

The behaviors of primary interest were shoving and ano-genital nuzzling. "Shoving" has been defined by Lacey, Alexander, Braude, Sherman, and Jarvis (1991) as an agonistic behavior in which two animals stand face to face, with their heads together, and one animal pushes the other backward for some distance. More than any other behavior, shoving has been implicated as the behavior that mediates reproductive suppression (Reeve and Sherman, 1991). Reeve and Sherman (1991) found that breeding females initiated more shoves than did any other colony member. Ano-genital nuzzling (Lacey et al., 1991) is a behavior that typically involves the breeding animals. The breeding female lies in a head-to-tail position with another animal (usually a breeding male), and the animals engage in mutual ano-genital licking, sniffing, and biting. Ano-genital nuzzling may thus be used as an index of reproductive status. Data were collected using an OS-3 event recorder (Gagetalker Corporation) and transferred directly to computer for analysis. The number of shoves given and received, the frequency of ano-genital nuzzling, and the identity of interactants were recorded for all subjects.

Urine Sample Collection and Hormone Assays

Urine collection. In conjunction with behavioral observations, urine samples were collected in order to assess the reproductive state of nonbreeding females before and after removal of the queen. Urinary progesterone was measured as an indicator of ovulation or pregnancy. Sample collection was attempted at least once per week from 20 May, 1991 to 15 March, 1992, using one of two collection methods. The first method took advantage of the fact that naked mole-rats utilize a toilet chamber.

Prior to urine collection, the toilet chambers were cleaned thoroughly with distilled water. When an animal entered the toilet chamber and urinated, the sample was collected with a sterile pipet, stored in a 1-ml cryovial and immediately placed on ice. The toilet chamber was then cleaned again before another animal entered. Samples were kept on ice for no more than 3 hr and then stored at −70°C until subsequent assay. We collected from 2 to 11 samples per collection day. The second method, while more intrusive, allowed us to select those animals from which we wished to collect a sample. The target animal was briefly removed from the colony and placed on a glass plate that had previously been cleaned thoroughly with distilled water. A moist cotton swab was used to stimulate the animal's ano-genital area. The sample was collected and stored as before, and the plate was then cleaned prior to collection of another sample. We were successful in obtaining urine in this manner on approximately half the attempts. Urine samples were obtained from 20 to 21 females in the colony, with a range of 1 to 17 samples per animal.

Creatinine assay. The creatinine concentration of each urine sample was assessed using a microtiter plate assay adapted from the method described by French, Abbott, Scheffler, Robinson, and Goy (1983). Urinary progesterone concentrations were expressed as mass/mg creatinine (mg Cr) to control for variations in the volume and concentration of the voided urine (Hodges and Eastman, 1984).

Progesterone assay. Progesterone in naked mole-rat urine was measured using a modification of the heterologous enzyme immunoassay procedure described by Saltzman, Schultz-Darken, Scheffler, Wegner, and Abbott (1994). Urine samples (500 μl) were extracted with 5 ml petroleum ether before duplicate aliquots of 200 μl were taken for assay. Procedural losses during extraction were estimated by the addition of known amounts (2000 cpm/100 μl) of [^{3}H]progesterone (sp act 115.0 Ci/mmol) to three additional samples per assay. These external recoveries were 84.1 ± 1.3% (mean ± SEM; N = 16). Serial dilutions of naked mole-rat urine (0.39–100.0 μl (N = 9)) gave displacement curves parallel to that obtained with progesterone standards (2.5–500 pg; Sigma, St. Louis, MO). The recovery of progesterone standards added to 100.0 μl of a naked mole-rat urinary pool (21.8 ± 2.3 pg/well) was 107.6 ± 3.3%. The sensitivity of the assay was 4.5 pg, and the intra- and interassay coefficients of variation were 4.0% (N = 8 assays) and 19.2% (N = 8 assays).

Urinary progesterone concentrations were only taken as indicative of luteal function or pregnancy when they exceeded 2 standard deviations (4.60 ng P/mg Cr) above the mean value (1.47 ng P/mg Cr) from all non-breeding females combined (excluding female 8; N = 82 samples from 16 females) before queen removal and after emergence of a new queen following conclusion of the present study.

Histopathology

Sixty-seven days after removal of the breeders from the colony, three females died as a result of intracolonial aggression. The reproductive tracts of these females were examined for signs of reproductive activity. Following gross examination and measurement of reproductive tracts, the tissues were fixed in 10% formalin, parafinized, and sectioned into 4-μm sections. The sections were deparafinized, mounted and stained with hematoxylin and eosin.

Data Analysis

The data were divided into three periods for analysis. The pre-removal period began 78 days before removal of the breeders. A total of twenty 10-min observations on each of 12 focal females was performed during this time. One focal female died during the pre-removal phase and was excluded from the study. On 6 September, the queen, breeding male, two non-breeding females, and two non-breeding males were removed from the colony. On 12 and 13 November, beginning 66 days following removal of the breeders, fighting broke out, culminating in the deaths of three females. The second period of observation covers the time from queen removal through the fighting, during which we collected a total of twenty 10-min observations on each of 10 focal females. The final period covers the time from the deaths of the first three females through the termination of behavioral observation, on 27 February 1992, 174 days after removal of the breeders. This final period again includes twenty 10-min observations on each of 5 focal females. For some analyses, this last period was further subdivided into two periods, of 12 and 8 observations, respectively, before and after a fight between two focal females. The number of focal animals declined during the course of the study due to (1) removal of animals ($N = 1$); (2) death of animals ($N = 4$); and (3) elimination of non-interactive animals during the final period of observation, after the fight between the two focal females ($N = 2$; animals 4 and 20).

Behavioral frequencies are presented as number of occurrences per 10 minutes. An F test for rates (as described by Cox and Lewis, 1966) was used to assess differences in shoving and ano-genital nuzzling rates of individual females from one phase of the study to the next. In order to analyze those cases in which rates were zero during a study period, we increased rates of zero to one behavior per study period (i.e., per 200 min). This provided a conservative underestimate of the magnitude of the change in rate. Differences among females in frequencies of shoving and ano-genital nuzzling were analyzed using a G test (Sokal and Rohlf, 1981).

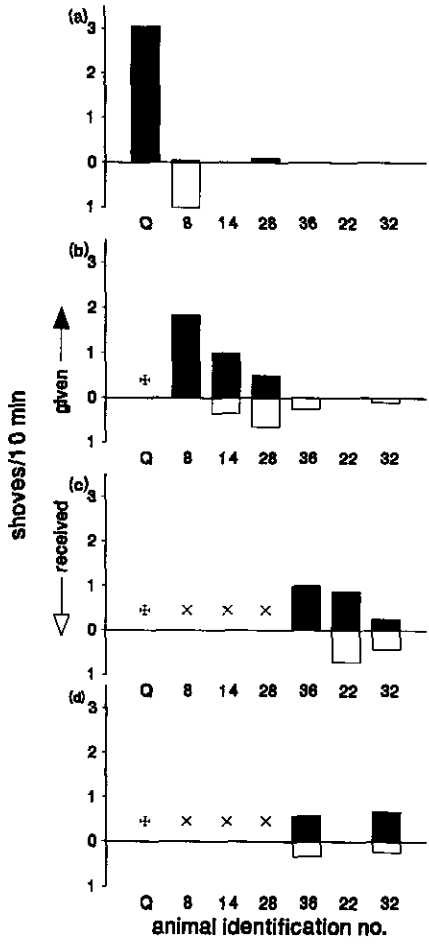


FIG. 1. Shoves given and received by selected females during the four phases of the study: (a) before queen's removal, 20 June–6 September, 1991; (b) after queen's removal, before fighting, 9 September–14 November, 1991; (c) after first fighting and deaths of 8, 14, and 28, 21 November–4 January, 1992; (d) after second fighting, 8 January–26 February, 1992. A cross indicates an animal that has been removed from the group. An x indicates an animal that has died. Shoves given are above the *x* axis; shoves received are below the axis.

RESULTS

Behavior

Shoving rates (mean shoves per 10 min) during each phase of the study are presented in Fig. 1. During the pre-queen removal period, the queen performed 95.4% of all observed intrasexual shoves by females, with females 8 and 28 accounting for the remaining 4.6%. The primary recipient

of shoves by the queen was female 8. She received 87.0% of all shoves to females and was shoved significantly more often by the queen than was any other female ($G = 32.2$, $P < 0.001$, $df = 2$). During the second time period, after the queen's removal, female 8 became the primary shover. She performed 56.8% of all observed shoves by females. Females 14 and 28 were responsible for all remaining shoves (29.7% and 13.5%, respectively). These three females shoved significantly more than any other females ($G = 92.6$, $p < 0.001$, $df = 6$). Female 8 directed 89.2% of her shoves towards females 14 and 28, more than towards any other animals ($G = 9.7$, $P < 0.05$, $df = 3$). All three females shoved significantly more after the queen was removed than before the queen was removed (F test, $F = 37.0$, $P < 0.001$, $df = 19,19$ for female 8; $F = 20.0$, $P < 0.001$, $df = 19,19$ for female 14; $F = 5.0$, $P < 0.002$, $df = 19,19$ for female 28). None of the 8 other focal females was observed to shove at all during this phase of the study, and only those females that were observed to shove at least once during the study were included in the analyses of changes in shoving rates.

Sixty-seven days after queen removal, female 28 attacked and killed female 8. The next day, females 28 and 14 were found dead, apparently having killed each other. No other animals were involved or injured in the fighting. Six days after the deaths of these three animals, female 32 was observed to shove for the first time, followed the next day by the first observed shoving by females 36 and 22 (see figure 1). Following the deaths of females 8, 14, and 28, female 22 was responsible for 14.3% of all shoves performed by females, female 32 for 40.0%, and female 36 for 45.7%. All three females shoved significantly more after the deaths of females 8, 14 and 28 than before their deaths (F test, $F = 31.6$, $P < 0.001$, $df = 19,19$ for female 36; $F = 17.6$, $P < 0.001$, $df = 19,19$ for female 22; $F = 18.4$, $P < 0.001$, $df = 19,19$ for female 32). These females shoved significantly more than any other females during this period ($G = 17.5$, $P < 0.01$, $df = 4$). Females 32 and 22 fought 52 days after the deaths of females 8, 14, and 28, and 119 days after the removal of the queen. Neither animal sustained serious injuries during the fight, in which the females locked incisors for a period of approximately 20 min while in a glass tube. Female 22 stopped shoving after the fight with female 32 ($F = 17.6$, $P < 0.002$, $df = 11,7$, compared to before the fight), and no longer received shoves. The shoving rates of females 32 and 36 did not change significantly following the fight ($F = 1.72$, $P < 0.5$, $df = 11,7$ for female 36; $F = 2.68$, $P < 0.2$, $df = 11,7$ for female 32).

Prior to the queen's removal, the queen was responsible for all observed ano-genital nuzzling by females. After the queen's removal, female 8 performed 40% of all observed ano-genital nuzzling, female 28 30%, and female 14 20% (Fig. 2). Female 4 performed the remaining 10% of nuzzling ($N = 1$ nuzzle). Females 8 and 28 ano-genital nuzzled significantly

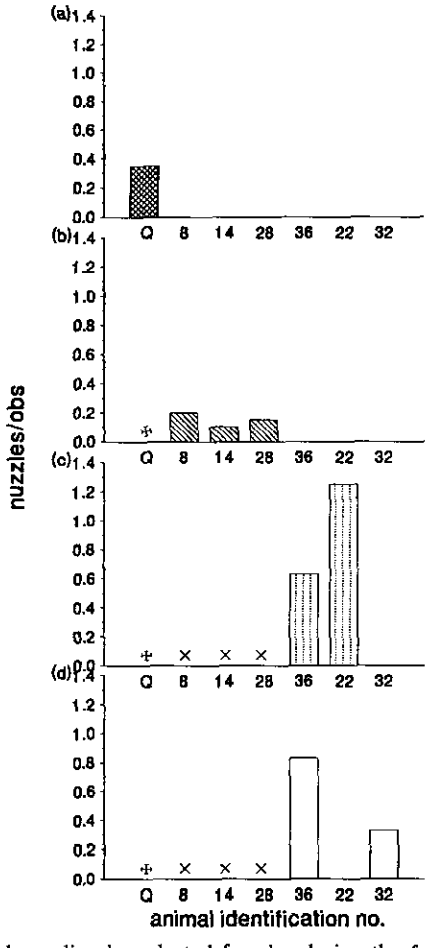


FIG. 2. Ano-genital nuzzling by selected females during the four phases of the study. Panels (a) through (d) as in Fig. 1.

more after removal of the queen (F test, $F = 4.0$, $P < 0.01$, $df = 19,19$ for female 8; $F = 3.0$, $P < 0.02$, $df = 19,19$ for female 28, $F = 2.0$, $P < 0.2$, $df = 19,19$ for female 14), but their nuzzling rates did not differ significantly from those of other females ($G = 0.68$, $df = 2$). After the deaths of females 8, 14, and 28, female 22 performed 28.2% of all ano-genital nuzzling by females, female 32 18.8%, and female 36 53.1%. Prior to the fight between females 22 and 32, females 22 and 36 ano-genital nuzzled significantly more after the deaths of females 8, 14, and 28 than before ($F = 12.6$, $P < 0.002$, $df = 19,11$ for female 36; $F = 25.0$, $P < 0.001$, $df = 19,11$ for female 22). After the fight between females 22 and 32, female 22 was no longer observed to engage in ano-genital nuzzling.

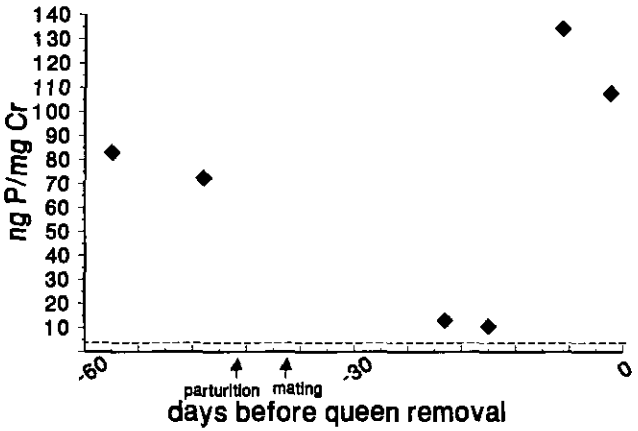


FIG. 3. Urinary progesterone levels of breeding female. Dashed line indicates 2 SD above the mean progesterone level of non-breeders (4.60 ng P/mg Cr).

Female 36 did not change her ano-genital nuzzling rate following the fight ($F = 1.32$, $P < 0.5$, $df = 11,7$); however, female 32 significantly increased her ano-genital nuzzling rate ($F = 6.6$, $P < 0.02$, $df = 11,7$). When these two periods after the deaths of females 8, 14, and 28 are combined, females 22, 32, and 36 ano-genital nuzzled significantly more than any other females ($G = 6.78$, $P < 0.05$, $df = 2$).

Hormones and Histopathology

Before she was removed from the colony, the queen exhibited a clear pattern of urinary progesterone concentrations (Fig. 3). Prior to parturition, progesterone levels were high, ranging from 72.27 to 82.89 ng P/mg Cr, values typical of mid- to late pregnancy (Faulkes *et al.*, 1991). Following parturition and subsequent mating, progesterone levels progressively rose from 10.65 to 134.18 ng P/mg Cr, typical of early to mid-pregnancy. During this latter period, female 8 had one high progesterone value of 32.6 ng P/mg Cr (Fig. 4). Females 14 and 28 exhibited some indication of ovarian activity before queen removal (urinary progesterone values exceeded our criterion level of 4.60 ng P/mg Cr in 4 out of 12 samples from both females combined). No other females exhibited such high urinary progesterone concentrations. The progesterone level of females 8, 14 and 28 prior to queen removal was 12.40 ± 5.82 ng P/mg Cr (mean ± SEM), significantly greater than the mean for the remaining 13 females from which we obtained urine samples during this period: (2.00 ± 0.09 ng P/mg Cr; Mann–Whitney U test, $U = 35$, $p < 0.05$).

The reproductive tracts of females 8, 14, and 28 were examined histologically, following the deaths of these three females in the colony. The

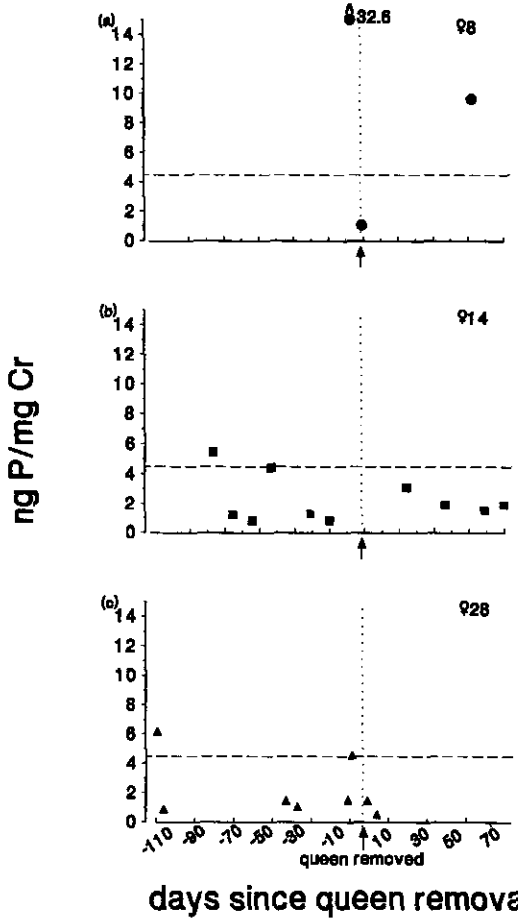


FIG. 4. Urinary progesterone levels of (a) female 8, (b) female 14, and (c) female 28. The date of queen removal (Day 0) is indicated on the x axis and by the dotted line. Dashed line indicates 2 SD above the mean (4.60 ng P/mg Cr). Values above 2 SD are considered to represent post-ovulatory, luteal phase activity.

size and mass of the ovaries and uterine horns of all 3 of these females were suggestive of reproductive activity (Table 1). In addition, females 8 and 28 had corpora lutea in their ovaries, along with primary and secondary follicles at varying levels of development (figures 5a and 5b). Female 14 had no corpora lutea; however, she did exhibit some follicular development (Fig. 5c).

Females 22, 32, and 36 exhibited no obvious ovarian activity (urinary progesterone <4.60 ng P/mg Cr) prior to the deaths of females 8, 14, and 28 (Fig. 6). After this point, female 22 showed signs of ovarian cyclicity

TABLE 1
Physical Characteristics of the Reproductive Tracts of Three Naked Mole-Rat Females

Female	Ovary mass (g)[a]	Reproductive tract mass (g)	Uterine horn length (mm)[b]
8	.045/.078	0.632	40/30
14	.050/.045	0.536	39/35
28	.069/.059	0.954	53/48
Breeders[c]	0.035 ± 0.11	1.25 ± 0.42	
Nonbreeders[c]	0.008 ± 0.002	0.042 ± 0.002	

[a] Mass of each ovary is presented separately.
[b] Length of each uterine horn is presented separately.
[c] Values from Faulkes (1990), based on $n = 4$ breeders and $n = 14$ non-breeders.

(urinary progesterone >4.60 ng P/mg Cr), which ceased after the fight with 32. Female 36 exhibited elevated concentrations of urinary progesterone (>4.60 ng P/mg Cr) after the fight between 32 and 22, indicative of her first pregnancy. Female 36 successfully produced and reared that litter, with no additional aggression or interference on the part of female 32 or female 22.

Weight and Age

The weights and ages of the 12 focal females are presented in Table 2. Females 8, 14, and 28 were all full sibling littermates. Females 8 and 28 were also the largest non-breeding females in the colony prior to removal of the queen. Of the three females who displayed signs of reproductive activity after the deaths of females 8, 14, and 28, females 22 and 32 were among the youngest animals in the colony, having been born during 1990, while female 36 was among the oldest animals. Female 36 was the only female in an otherwise all-male litter. Female 32 was the largest animal in the colony at this time; however, both females 22 and 36 were smaller than female 16, a female which did not show any signs of reproductive activity.

DISCUSSION

In this first description of female succession to breeding status in a naked mole-rat colony, we found signs of ovarian activity among non-breeding females in a colony with an already actively breeding queen present. Non-breeding females typically did not exhibit characteristic queen-like behaviors (shoving and ano-genital nuzzling) while in the presence of a reproductively active female (female 8 was observed to shove only once prior to removal of the breeding female; female 28 twice). However, one female (female 8) was the primary target of shoving by

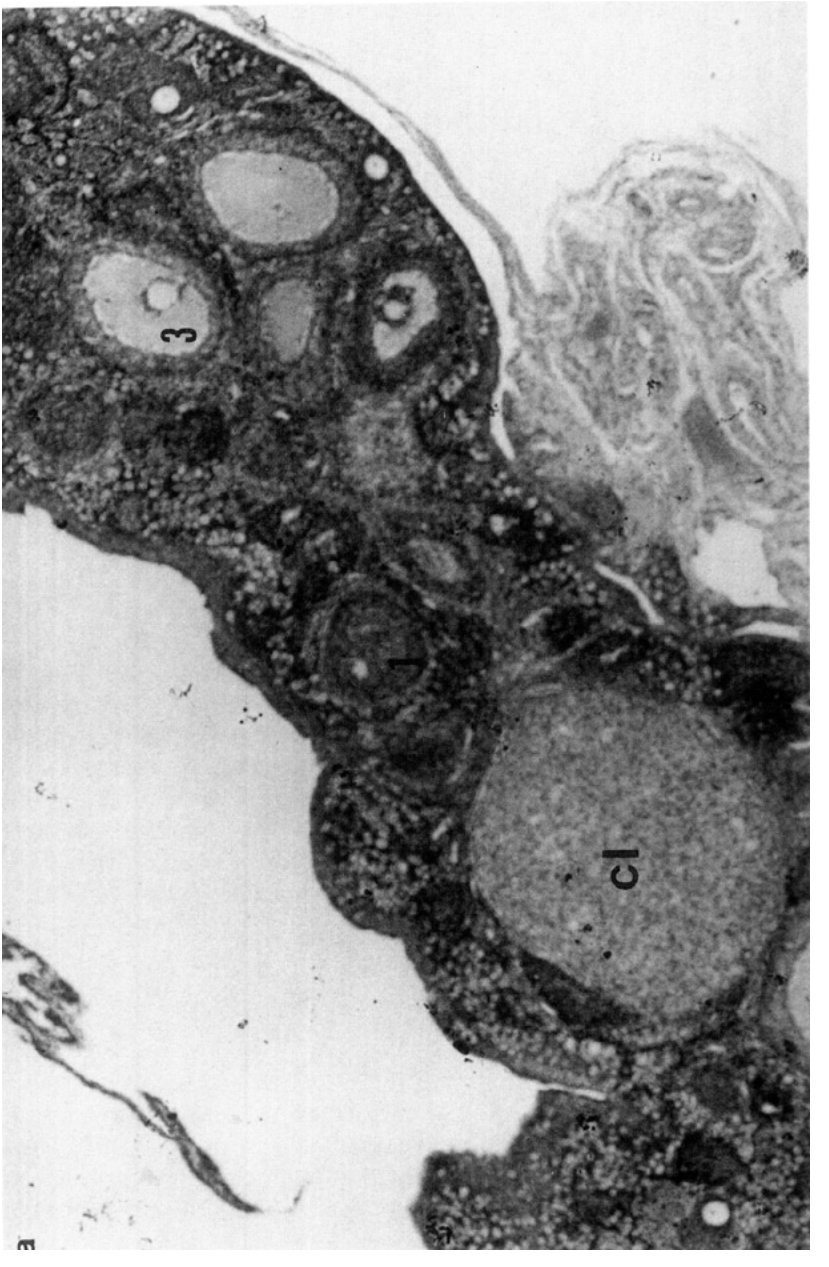
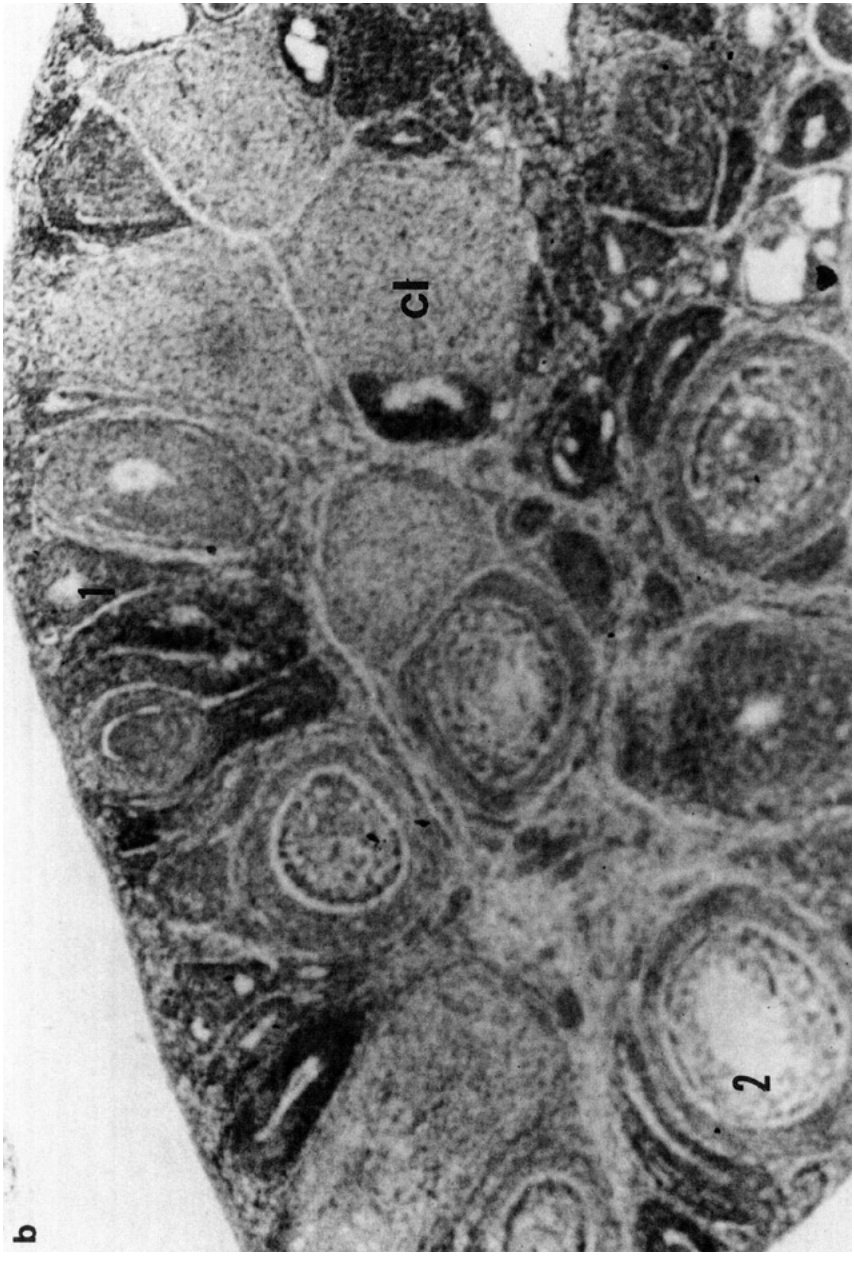


FIG. 5. Cross-section of one ovary of (a) female 8, (b) female 28, and (c) female 14. Corpora lutea (cl), primary follicles (1), secondary follicles (2), and tertiary follicles (3) are indicated where appropriate on each photo.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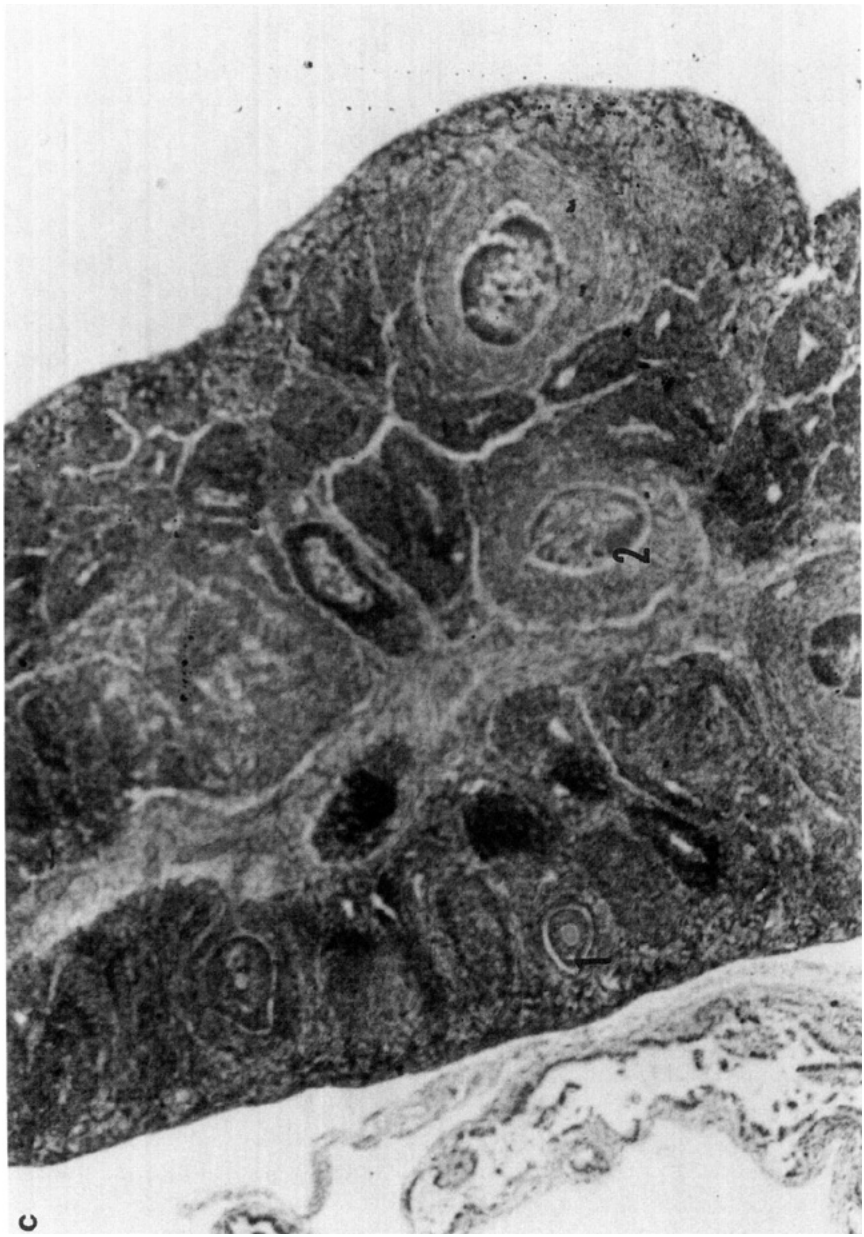

FIG. 5—*Continued*

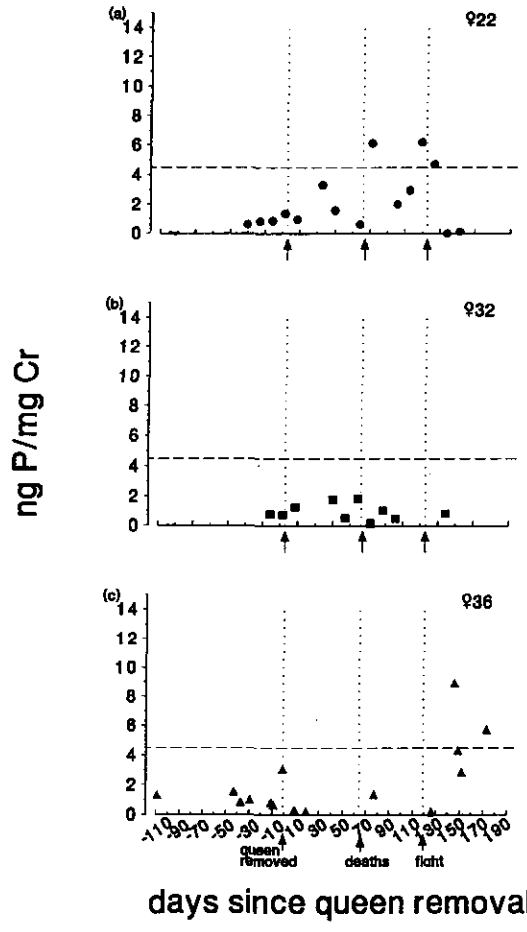

FIG. 6. Urinary progesterone levels of (a) female 22, (b) female 32, and (c) female 36. The dates of queen removal (Day 0), the deaths of females 8, 14, and 28 (Day 67), and the fight between 32 and 22 (Day 124) are indicated on the *x* axis and by the dotted line.

the queen. This same animal was one of three females found to exhibit hormonal signs of ovarian activity while in the presence of the breeding female, and was the first female to exhibit characteristic queen-like behaviors shortly after the removal of the queen from the colony.

The results of this study indicate that non-breeding females differ in their likelihood of actively competing for breeding status following queen removal. Of the 18 females remaining in the colony after queen removal, only three began to exhibit queen-like behaviors and hormonal indications of ovarian cyclicity. Following the deaths of these three females, similar changes were apparent in only three of the remaining 15 females. Thus, whereas some non-breeding female naked mole-rats rapidly escape be-

TABLE 2
Ages and Weights of Focal Females

Female	Age at queen removal[a] (months)	Weight (g) at queen removal	Weight on 29 Nov. 1991[b]	Weight on 25 Jan. 1992[c]
Queen	68	73.2	—	—
8	35	52.6	—	—
14	35	41.5	—	—
28	35	45.7	—	—
22	10–20[d]	40.5	41.0	47.0
32	10–20[d]	43.8	51.0	58.0
36	41	42.6	45.0	48.0
2	35	39.0	40.5	42.0
4	10–20[d]	34.3	32.0	38.0
16	35	41.9	46.0	50.0
20	10–20[d]	34.6	35.0	35.0

Note. Focal female 12 died prior to queen removal and is excluded from the table.
[a] Queen removal occurred on 6 September, 1991.
[b] The deaths of females 8, 14, and 28 occurred on 12 and 13 November, 1991.
[c] The fight between females 32 and 22 occurred on 3 January, 1992.
[d] Three litters were born between January and November of 1990. Animals were not individually marked at this time and cannot be reliably assigned to a particular litter.

havioral and hormonal suprression following queen removal and appear to compete for breeding status, others fail to do so. The sources of these individual differences are unknown. As has been reported previously by Jarvis (1991a), we found no consistent pattern with respect to age or weight of females competing to become the new queen; however, the oldest female in the colony emerged as the new queen (female 36). Those females who vied for breeding status were among the largest females in the colony, but not all large females participated in the struggle for reproductive dominance.

Prior to her removal, the queen preferentially directed her shoving towards female 8, the female to show the most obvious signs of reproductive activity. The observed elevated levels of progesterone indicated that ovarian cycles were probably occurring in female 8 and possibly in females 14 and 28. The histological results supported the behavioral and endocrinological assessment of reproductive activity in two of these females, 8 and 28.

Comparable behavioral and hormonal changes were observed in females 22, 32 and 36 following the deaths of females 8, 14, and 28. The fight between females 22 and 32 offers an opportunity for speculation. Prior to this time, female 22 showed both hormonal and, to a lesser degree, behavioral signs of reproductive activation. After the fight between fe-

males 22 and 32, female 22 dramatically decreased her shoving rate and ceased to exhibit urinary progesterone concentrations indicative of luteal function. Female 36 had shown elevated rates of shoving for at least 43 days preceding this fight, and although she was not involved in the fighting, she began to exhibit elevated progesterone levels following the fight. The fight between female 32, which had shown no signs of ovarian cyclicity, and female 22, which exhibited apparent cyclicity, resulted in a cessation of ovarian activity in female 22. Furthermore, the onset of ovarian cyclicity of female 36 coincided with the fight between females 32 and 22. The fight between females 32 and 22 appears to have served as a proximate trigger for the ovarian activation of female 36, as well as a cue for cessation of ovarian activity in female 22.

As has been previously reported by Jarvis (1991a), we found that the intra-colonial events leading up to queen succession varied, from peaceful and rapid to slow with severe fighting. Regardless of the pattern of events leading up to queen succession, we did find hormonal and histopathological indications of likely reproductive activity in specific non-breeders while still in the presence of the queen, which has not been previously reported. However, Jarvis (1991a) has reported the presence of multiple breeding females in several captive colonies. In such cases, pup survival is usually poor and the situation eventually terminates violently. Additionally, we found evidence that the queen was able to detect these indications of potential reproductive activity, and preferentially directed her aggressive shoves towards these females.

Pheromones are known to play a role in reproductive suppression, activation, and synchrony in a variety of species, including other cooperative breeders, such as the cotton-top tamarin (*Saguinus oedipus;* Savage, Ziegler, and Snowdon, 1988) and the common marmoset (*Callithrix jacchus;* Barrett, Abbott, and George, 1990), and a wide variety of rodent species (see for example, McClintock, 1987; Bronson, 1987). Naked mole-rats are known to wallow in the communal toilet chamber, presumably picking up characteristic colony odors that may be present in the urine (Lacey et al., 1991). However, possibly pheromones present in the urine do not appear to play a role in mediating reproductive suppression in naked mole-rats (Faulkes and Abbott, 1993). We have no data in our study to indicate whether toilet chamber visitation rates changed after removal of the queen.

Behaviorally mediated reproductive suppression is seen in a number of other species. A close parallel to our findings in naked mole-rats may be found in the common marmoset. Saltzman, Schultz-Darken, and Abbott (in preparation) found that dominant females in newly formed captive groups directed their aggression preferentially towards those subordinate females that had previously undergone cyclic ovarian activity. A similar situation is found in free-living gelada baboons (*Theropithecus gelada*)

(Dunbar, 1989) and yellow baboons (*Papio cynocephalus*) (Wasser and Starling, 1988). In both latter species, low-ranking females show markedly reduced reproductive success, presumably as a result of the aggression they receive from dominant females. It appears that naked mole-rats may present another case of preferential aggression towards cycling, subordinate females. Still another example may be found in the sequentially hermaphroditic fishes (Demski, 1987), in which aggression by the breeding male toward the dominant female prevents sex change in the female (Robertson, 1972). The dominant female in turn prevents sex change in lower-ranking females through aggressive behavior.

In conclusion, hormonal changes indicative of ovarian cyclicity were observed to occur in several non-breeding naked mole-rat females both before and following removal of the breeding female. The behavior of the queen that is believed to mediate reproductive suppression, aggressive shoving, was directed preferentially towards one of those females who subsequently displayed the first signs of reproductive activation. Behavior of subordinate females seemed unlikely to play a major role in eliciting queen aggression, because non-breeding females shoved and ano-genital nuzzled at very low frequencies while in the presence of the breeding female. However, this possibility cannot be completely excluded. Some non-behavioral indication of ovarian activation among specific non-breeders, such as scent, may be the proximate cue by which the breeding female identifies likely competitors for breeding status. Whether or not the ovarian activity of non-breeding females triggers aggressive behavior on the part of the queen remains to be determined; however, it is clear that naked mole-rat females are able to monitor the reproductive status of other individuals and that non-breeding females may infrequently undergo ovarian activation while in the presence of a queen. Thus even during a period of apparent stability in a colony, the queen must actively maintain reproductive suppression of subordinate females or risk either being supplanted or having multiple females produce litters (Jarvis, 1991a).

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